STIC-ILL

QR 185.8.H93

From: STIC-Biotech/ChemLib
Sent: Thursday, March 18, 2004 5:01 PM
To: STIC-ILL
Subject: FW: 09899300

-----Original Message-----
From: Yaen, Christopher
Sent: Thursday, March 18, 2004 4:58 PM
To: STIC-Biotech/ChemLib
Subject: 09899300

could you please get the following ref(s):

Br J Cancer. 1998 Aug;78(4):478-83

J Immunother Emphasis Tumor Immunol. 1996 Jul;19(4):245-56.

Hybridoma. 1986 Jul;5 Suppl 1:S117-23.

Med Oncol Tumor Pharmacother. 1986;3(3-4):141-6.

Hybridoma. 1986 Jul;5 Suppl 1:S163-70.

Hybridoma. 1986 Jul;5 Suppl 1:S151-61

Hybridoma. 1986 Jul;5 Suppl 1:S125-32.

Hybridoma. 1986 Jul;5 Suppl 1:S175-83.

Christopher Yaen
US Patent Office
Art Unit 1642
571-272-0838
REM 3A20
REM 3C18

HYBRIDOMA
Volume 5, Suppl. 1, 1986
Mary Ann Liebert, Inc., Publishers

Phase I Clinical Trial of CO17-1A Monoclonal Antibody

ALBERT F. LOBUGLIO,[1] MANSOOR SALEH,[1] LYNN PETERSON,[1] RICHARD WHEELER,[1] RICHARD CARRANO,[2] WILLIAM HUSTER,[1] and M.B. KHAZAELI[1]

ABSTRACT

Twenty patients with metastatic gastrointestinal cancer received one or more weekly infusions of 400 mg CO17-1A monoclonal antibody. The most common side effect was mild gastrointestinal symptoms in 9/20 patients. Two of five patients receiving three weekly infusions had reversible anaphylactic reactions at the time of their third infusion. The pharmacokinetics of the antibody were similar at the first, second or third infusion. Human antibody to 17-1A occurred in 17/20 patients with 11/20 having antibody detectable by 8 days following initial infusion. Thus, one or two infusions (weekly) of large doses of 17-1A were well tolerated but allergic responses limit ability to administer therapy by 15 days post-initial infusion.

INTRODUCTION

The murine monoclonal antibody designated CO17-1A (17-1A) was produced by investigators at the Wistar Institute (1) and has been extensively characterized in regard to its specificity and *in vitro* biologic activity (2,3). In nude mice carrying colon cancer cell line implants, it has been shown to localize to the tumor site and to have *in vivo* anti-tumor activity (4). Phase I studies in man have suggested that it is well tolerated and may have beneficial *in vivo* effects in patients with gastrointestinal malignancies (5). We have carried out a Phase I/II trial in 20 patients to examine patient tolerance to repeated high doses of 17-1A, examine its pharmacokinetics on repeated administration and to characterize the human immune response (antibody) to this mouse immunoglobulin.

[1]Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL 35294; [2]Centocor, Inc., Malvern, PA 19355

MATERIALS AND METHODS

Patient Population

Twenty patients with gastrointestinal malignancy (17 colon; 2 gastric; and 1 pancreatic) were selected on the basis that they had metastatic disease with small-moderate tumor burden; performance status >70% (Karnofsky scale) and objectively measurable disease. Studies were not done to document 17-1A reactivity with individual patient tumor specimens. Seven out of 20 patients had received prior chemotherapy while 13/20 had no prior therapy for metastatic disease.

Treatment Protocol

All antibody infusions utilized a total dose of 400 mg of 17-1A diluted in 250 ml of normal saline infused over 30 minutes in our Clinical Research Unit with careful monitoring of vital signs. All infusions were preceded by an intravenous test dose of 0.7 mg followed by 30 minutes of monitoring prior to administration of the full dose infusion. The protocol involved the accrual of 4 groups of 5 patients each who would receive progressively increasing numbers of weekly infusions, i.e. Group 1 - 5 patients - single infusion; Group 2 - 5 patients with 2 infusions - Day 1 and 8; Group 3 - 5 patients with 3 infusions - Day 1, 8 & 15; Group 4 - 5 patients with 4 infusions - Day 1, 8, 15 & 22. Because of toxicity noted in Group 3 patients, no patients received 4 infusions, but these 5 patients were added to Group 2 and had 2 infusions of therapy (Day 1 & 8). All patients were followed for 6 weeks, following their last infusion with weekly monitoring of urinalysis, liver and renal function, blood counts and clinical evaluation.

Pharmacokinetics

The pharmacokinetics were done on the first 5 patients at the time of their only infusion (no prior exposure to 17-1A), the ten patients who had 2 infusions were studied at the time of their second infusion (one prior exposure to 17-1A) and the group 3 patients were studied at the time of their third infusion (two prior exposures to 17-1A). For pharmacokinetics, blood samples were drawn prior to infusion, immediately at conclusion of infusion and at ½, 1, 2, 4, 12, 24, 48, 72 hours and 96 hours. Spot samples were drawn at pre-therapy, 1, 24 & 48 hours post-therapy on infusions not having a full pharmacokinetic study to confirm the general pattern of mouse immunoglobulin disappearance. The plasma level of 17-1A was quantitated using a solid phase radiometric sandwich assay utilizing latex beads coated with rabbit anti-mouse gammaglobulin and radiolabeled (^{125}I) affinity purified goat anti-mouse IgG, $F(ab')_2$. The concentration of 17-1A in plasma was quantitated by the amount of latex particle binding of radiolabeled anti-mouse IgG, $F(ab')_2$ as compared to a standard curve of known concentrations of 17-1A diluted in normal plasma. The sensitivity of this assay was 1.0 ng/ml.

Human Anti-mouse Antibody (HAMA) Response

Serum samples were drawn on each patient prior to each infusion and then weekly x 6. The assay used to determine the presence of human anti-17-1A was a "double antigen" system (6) using the concurrent incubation of 17-1A coated latex beads, 100 ul of test plasma and 1 ug of radiolabeled (^{125}I) 17-1A (specific activity of 300-400 cpm/ng). The samples were incubated 90 minutes at room temperature and the radioactivity associated with the beads determined by centrifugation of the beads through Percoll as previously described (7). The cpm of ^{125}I-17-1A bound to the beads by plasma was converted to ng of 17-1A/ml of plasma by using the known

ETHODS

tinal malignancy (17 colon; ected on the basis that they erate tumor burden; perform- and objectively measurable cument 17-1A reactivity with Seven out of 20 patients had 20 had no prior therapy for

a total dose of 400 mg of ine infused over 30 minutes areful monitoring of vital an intravenous test dose of ring prior to administration ol involved the accrual of uld receive progressively i.e. Group 1 - 5 patients - ith 2 infusions - Day 1 and s - Day 1, 8 & 15; Group 4 - 8, 15 & 22. Because of patients received 4 infu- to Group 2 and had 2 infu- atients were followed for with weekly monitoring of blood counts and clinical

he first 5 patients at the xposure to 17-1A), the ten ied at the time of their o 17-1A) and the group 3 heir third infusion (two cokinetics, blood samples at conclusion of infusion nd 96 hours. Spot samples urs post-therapy on infu- ic study to confirm the lisappearance. The plasma solid phase radiometric ed with rabbit anti-mouse affinity purified goat on of 17-1A in plasma was le binding of radiolabeled standard curve of known plasma. The sensitivity

ient prior to each infu- to determine the presence en" system (6) using the ex beads, 100 ul of test 1A (specific activity of ated 90 minutes at room ed with the beads deter- gh Percoll as previously d to the beads by plasma sma by using the known

specific activity of ^{125}I-17-1A. This assay obviously detects any molecule with more than one binding site for 17-1A (IgG and IgM). Assay results in normal individuals and cancer patients prior to 17-1A exposure were 5 ± 4 ng/ml plasma (n=54) with values ranging from 0 to 16 ng/ml. Values >20 ng/ml were classified as an antibody response.

17-1A Monoclonal Antibody

The monoclonal antibody was provided by Centocor, Inc. as a purified suspension of 10 mg/ml in normal saline. It was stored at 4°C prior to use. The protocol was carried out under Centocor sponsored IND (#2168).

RESULTS

Toxicity

The adverse effects of 17-1A administration are summarized in Table 1. Ten of 20 patients had no adverse effects including 4 patients who received two infusions and 3 patients who received three infusions. The most frequent side effect was gastrointestinal (9/20 patients) with nausea and vomiting (4 patients) or diarrhea with or without cramps (7 patients). The symptoms usually began within an hour of infusion and lasted <24 hours. They were of modest-moderate severity and readily controlled with antiemetics or anti-diarrhea medications. The frequency of gastrointestinal symptoms was not related to the number of 17-1A infusions. One patient had an episode of flushing and tachycardia in the midst of her second infusion which disappeared by simply slowing the infusion rate. She had no other adverse effects with the infusion nor with her prior infusion.

Two patients had serious adverse effects. Both patients had nausea and vomiting associated with their first and second infusions (Day 1 & 8). They tolerated their test dose of 17-1A on Day 15 without adverse effects over 30 minutes of observation. The treatment infusions were then begun and both developed dyspnea, tachycardia and hypotension judged to be an anaphylactic reaction. Both infusions were immediately stopped (less than 10% of dose given) and patients responded well to therapy with corticosteroids, epinephrine and antihistamines. No patient in the study developed abnormalities of urinalysis, complete blood count, renal or hepatic function.

Pharmacokinetics

The serial plasma 17-1A levels on each patient were analysed and found to fit well with a 1 compartment model of plasma disappearance. The results for peak plasma concentration, plasma half-life and area under the curve are summarized in Table 2. The two patients who had anaphylactic reactions did not receive their full third dose of 17-1A and therefore had no pharmacokinetic study. Thus, only 3 patients made up the group with two prior exposures to 17-1A. The results are similar for all 3 groups of patients. The three patients studied on their third infusion had a somewhat lower serum peak concentration of 17-1A and a somewhat longer mean plasma half-life than the groups of patients with a single or second infusion. Interpretation is limited since the differences were modest and the group was made up of a small number of patients.

Human Anti-mouse Antibody (HAMA) Response

The patients' serum prior to therapy had little or no detectable ability to bind ^{125}I-17-1A to 17-1A coated beads. As summarized in Table 3, almost all patients developed HAMA within 29 days of their first 17-1A exposure (17/20). The majority (11/20) had HAMA by Day 8 with 8/11 having values >100 ng/ml and 2/11 having

Table 1. TOXICITY ASSOCIATED WITH 17-1A INFUSION

I. Single dose (400 mg) - 5 patients

3/5 - none
2/5 - G.I. symptoms

II. Two weekly doses (400 mg) - 10 patients

4/10 - none
5/10 - G.I. symptoms
1/10 - flushing/tachycardia

III. Three weekly doses (400 mg) - 5 patients

3/5 - none
2/5 - G.I. symptoms and anaphylaxis (third dose)

Table 2. PHARMACOKINETICS 17-1A (400 mg) IN MAN

Prior Antibody	Peak Conc. (ug/ml)	Half-life (hours)	AUC (hrs-ug/ml)
None (n=5)	139 ± 8	15 ± 2	3013 ± 175
One (n=10)	141 ± 5	14 ± 1	2828 ± 93
Two (n=3)	108 ± 2	24 ± 2	3771 ± 81

Values are expressed as mean ± standard error of the mean. AUC = area under the curve.

Table 3. HUMAN ANTI-MOUSE ANTIBODY (HAMA) RESPONSE*

Pre-therapy	- 0/20 had antibody (range - 0-15 ng/ml)
Day 8	- 11/20 had antibody (range - 36-1106 ng/ml)
Day 15	- 15/20 had antibody (range - 27-5598 ng/ml)
Day 22	- 14/20 had antibody (range - 60-5046 ng/ml)
Day 29	- 15/20 had antibody (range - 23-4900 ng/ml)
No antibody	- 3/20

*Antibody activity expressed as ng of ^{125}I-17-1A bound/ml plasma.

WITH 17-1A INFUSION

ents

0 patients

dia

5 patients

anaphylaxis (third dose)

00 mg) IN MAN

Half-life (hours)	AUC (hrs-ug/ml)
15 ± 2	3013 ± 175
14 ± 1	2828 ± 93
24 ± 2	3771 ± 81

error of the mean. AUC =

HAMA) RESPONSE*

y (range - 0-15 ng/ml)

y (range - 36-1106 ng/ml)

y (range - 27-5598 ng/ml)

y (range - 60-5046 ng/ml)

y (range - 23-4900 ng/ml)

^{125}I-17-1A bound/ml plasma.

Table 4. DEGREE OF HUMAN ANTI-MOUSE ANTIBODY (HAMA) RESPONSE*

Exposures	Very High (>1000)		Moderate (40-999)		Poor/none (<40)	
Single	3	(60%)	1	(20%)	1	(20%)
Double	4	(40%)	4	(40%)	2	(20%)
Triple	2	(40%)	2	(40%)	1	(20%)

*Expressed as ng of 17-1A bound/ml plasma

values of >1000 ng/ml. Peak values were generally noted on Day 15 or 22 with values falling by Day 29 and beyond. Patients who received one, two or three exposures to 17-1A had similar degrees of HAMA response as summarized in Table 4.

The two patients with anaphylactic reactions were interesting. They had HAMA levels of 1055 and 264 ng/ml on Day 8 and 1716 and 3745 ng/ml on Day 15, respectively. They tolerated their infusions of antibody on Day 8 without adverse effect except for nausea and vomiting (similar to what they had on Day 1 infusion) but had anaphylactic reactions on Day 15 at the time of their third infusion. A total of 11 infusions were administered to patients when their HAMA levels were >20 ng/ml (elevated) with five having no side effects, three gastrointestinal symptoms, one flushing/tachycardia and two anaphylactic reactions. No patients developed fever, proteinuria or renal impairment.

It was also interesting that in nine of these 11 infusions, adequate plasma samples were available to determine peak plasma concentration and plasma half-life of 17-1A antibody. These values were not substantially different than infusions in the absence of detectable HAMA.

DISCUSSION

This phase I/II study of repeated administration of 400 mg 17-1A monoclonal antibody provides several observations. In general, the administration of antibody was well tolerated in patients receiving one or two infusions. The mild gastrointestinal symptoms were clearly related to antibody infusion and were not a serious clinical problem. The pathogenesis of these symptoms is not known but does not seem related to an allergic reaction since they occurred just as frequently during a patient's first infusion as compared to third infusion. They may be related to the ability of this antibody to bind to normal gastrointestinal mucosa (8). Two of five patients receiving three weekly infusions of 17-1A had anaphylactic reactions. This frequency of a potentially life-threatening allergic reaction precluded our testing a four-dose schedule (weekly) and would deter treatment schedules requiring antibody administration on Day 15.

The pharmacokinetic studies indicate that this dose of antibody can achieve plasma concentrations of 100-200 ug/ml with a plasma disappearance curve approximating observations with other mouse monoclonal antibodies (radiolabeled) administered at much lower dosages (9,10). This plasma half-life results in plasma concentrations of <1 ug/ml by day 8. Thus, maintenance of a substantial plasma concentration of 17-1A would require administration more frequently than weekly. Prior studies (9,11) have suggested that the appearance of HAMA response is associated with a dramatic alteration in circulating levels of mouse Ig. Our failure to observe this phenomenon is somewhat surprising. However, it should be noted that our antibody measurements are

expressed in terms of ng 17-1A bound/ml plasma with an infusion of 17-1A which readily achieves concentrations of 100-200 ug/ml in the circulation. We are currently modifying our HAMA assay to allow quantitation of total circulating HAMA. This may clarify whether the patient's total circulating HAMA is able to bind only a small fraction of this large circulating dose of 17-1A.

We did not find evidence of pre-existing human anti-mouse antibody (17-1A) prior to antibody infusion as reported by others (12). We initially attempted to assay for human anti-mouse antibody using an assay which detected human immunoglobulin binding to 17-1A coated beads using radiolabeled monoclonal mouse anti-human Fc antibody. We found that normal individuals and cancer patients prior to monoclonal antibody infusion had varying quantities of human immunoglobulin which bound non-specifically to 17-1A coated beads. This binding did not have classic competitive inhibition by soluble antigen (17-1A) and was judged to be a non-specific phenomenon and not antibody. In contrast, post-immunization plasma immunoglobulin binding to 17-1A coated beads was readily inhibited by soluble antigen (17-1A). Thus, we believe that the double antigen assay system used in this study more clearly reflects immune response to 17-1A. Despite the administration of one or more large doses of 17-1A, human antibody response to this protein was prompt with antibody frequently detectable by Day 8 and appreciable levels of antibody achieved by Day 15 & 22. Further studies are underway to characterize this antibody response in regards to immunoglobulin subclass and anti-idiotype.

ACKNOWLEDGMENTS

We would like to acknowledge the technical assistance of Bruce Thompson and manuscript preparation by Sharon Garrison and Kathy Walker.

REFERENCES

1. HERLYN, M., STEPLEWSKI, Z., HERLYN, D., and KOPROWSKI, H., (1979). Colorectal carcinoma specific antigen: Detection by means of monoclonal antibodies. Proc. Natl. Acad. Sci. USA 76, 1438-1442.

2. KOPROWSKI, H., STEPLEWSKI, Z., MITCHELL, K., HERLYN, M., HERLYN, D., and FUHRER, P., (1979). Colorectal carcinoma antigens detected by hybridoma antibodies. Somat. Cell Genet. 5, 957-972.

3. HERLYN, D., HERLYN, M., STEPLEWSKI, Z., and KOPROWSKI, H., (1979). Monoclonal antibodies in cell-mediated cytotoxicity against human melanoma and colorectal carcinoma. Eur. J. Immunol. 9, 657-659.

4. HERLYN, D., STEPLEWSKI, Z., HERLYN, M., and KOPROWSKI, H., (1980). Inhibition of growth of colorectal carcinoma in nude mice by monoclonal antibody. Cancer Res. 40, 717-721.

5. SEARS, H., HERLYN, D., STEPLEWSKI, Z., and KOPROWSKI, H., (1984). Monoclonal antibody immunotherapy. J. Biol. Resp. Modif. 3, 138-150.

6. ADDISON, G. and HALE, C., (1971). Two site assay of human growth hormone. Horm. Metab. Res. 3, 59-60.

LOBUGLIO, A., COURT, W., VINOCUR, L., MAGLOTT, G., and SHAW, G., (1983). Immune Thrombocytopenia Purpura: Use of a ^{125}I-labeled anti-human and anti-IgG monoclonal antibody to quantify platelet bound IgG. New Engl. J. Med. 309, 459-463.

8. SEARS, H., HERLYN, D., HERLYN, M., GROTZINGER, P., STEPLEWSKI, Z., GERHARD, W., and KOPROWSKI, H., (1981). Ex vivo perfusion of a tumor containing colon with monoclonal antibody. J. Surg. Res. 31, 145-150.

9. PIMM, M., PERKINS, A., ARMITAGE N., and BALDWIN, R., (1985). The characteristics of blood-borne radiolabels and the effect of anti-mouse IgG on localization of radiolabeled monoclonal antibodies in cancer patients. J. Nucl. Med. 26, 1011-1023.

10. ROSENBLUM, M., MURRAY, J., HAYNIE, T., GLENN, H., JOHNS, M., BENJAMIN, R., FRINKE, J., CARLO, D., and HERSH, E., (1985). Pharmacokinetics of ^{111}In-labeled anti-p97 monoclonal antibody in patients with metastatic malignant melanoma. Cancer Res. 45, 2382-2386.

11. LARSON, S., BROWN, J., WRIGHT, P., CARRASGUILLO, J., HELLSTRÖM, I, and HELLSTRÖM, K., (1983). Imaging of melanoma with I^{131}-labeled monoclonal antibodies. J. Nucl. Med. 24:123-129, 1983.

12. SCHROFF, R., FOON, K., BEATTY, S., OLDHAM, R., and MORGAN, A., (1985). Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. Cancer Res. 45, 879-885.

Address reprint requests to:

M.B. Khazaeli, Ph.D.
Comprehensive Cancer Center
223H Tumor Institute
University of Alabama at Birmingham
University Station
Birmingham, Alabama 35294